Nature Reviews Molecular Cell Biology | AOP, published online 17 June 2015; doi:10.1038/nrm4016

UNFOLDED PROTEIN RESPONSE

Regulatory ribosomal ubiquitylation

The ubiquitylation of misfolded proteins during the unfolded protein response (UPR) is known to target these proteins for degradation, but Bennett and colleagues now report in *Molecular Cell* that the ubiquitylation of 40S ribosomal proteins during the UPR has a role in the regulation of translation that is conserved across eukaryotes.

The authors used a quantitative proteomics approach together with enrichment for diGly-modified peptides (which are generated by trypsin-mediated cleavage of ubiquitylated proteins) in a human cell line stimulated with the UPR-inducing agents dithiothreitol (DTT) and tunicamycin. Treatment with DTT and tunicamycin led to specific changes in the abundance of a portion of diGly-modified peptides without inducing global changes in polyubiquitin chain abundance.

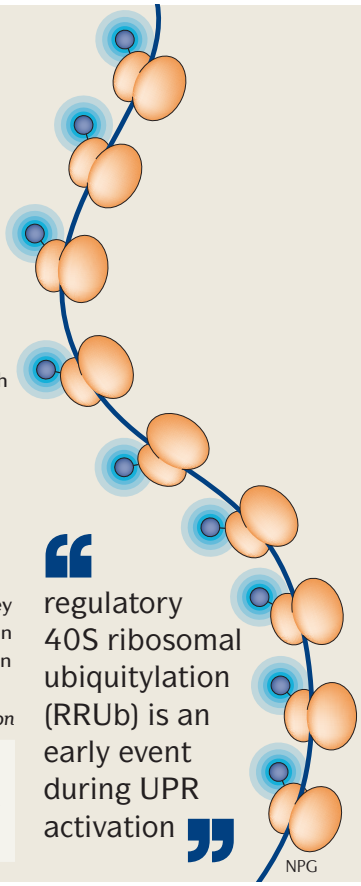
The ubiquitylated proteins affected by UPR induction were enriched for proteins involved in mRNA translation, such as 40S ribosomal subunits. Proteasome inhibition, which is known to result in the loss of Lys regulatory ubiquitylation marks, led to the deubiquitylation of specific Lys residues of RPS2, RPS3 and RPS20 proteins of the 40S ribosome. The same Lys residues in RPS2 and RPS3 were ubiquitylated within 30 minutes of DTT treatment, which shows that this regulatory 40S ribosomal ubiquitylation (RRUb) is an early event during UPR activation.

Translation inhibition during the UPR is mediated by phosphorylation of the translation initiation factor eIF2α by the endoplasmic reticulum (ER)-localized kinase PERK. Activation of PERK was necessary but not sufficient for UPR-induced regulatory ubiquitylation of RPS2 and RPS3, and further work is required to elucidate the upstream pathway that induces RRUb. The inability to decrease protein synthesis upon UPR activation has previously been shown to lead to increased cell death. In this study, mutating specific Lys residues in RPS2 and RPS20 to prevent regulatory ubiquitylation rendered cell lines expressing these mutant proteins more susceptible to induced cell death after UPR activation, which indicates that the observed RRUb does indeed regulate the cellular response during UPR activation.

The authors conclude that RRUb joins eIF2α phosphorylation as a second mechanism of UPR-induced translational control. Furthermore, they show that this regulatory ubiquitylation is conserved in a site-specific manner in yeast and fruitflies.

Kirsty Minton

ORIGINAL RESEARCH PAPER Higgins, R. *et al.* The unfolded protein response triggers site-specific regulatory ubiquitylation of 40S ribosomal proteins. *Mol. Cell* http://dx.doi.org/10.1016/j.molcel.2015.04.026 (2015)



> “regulatory 40S ribosomal ubiquitylation (RRUb) is an early event during UPR activation”

NPG